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Integrative transcriptome profiling and machine learning analysis reveal systemic immune signature of Bojungikki-tang with PD-L1 blockade in non–small cell lung cancer

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Bojungikki-tang, a traditional multi-herbal formula, has shown synergistic antitumor effects with immune checkpoint inhibitors in preclinical models. However, its systemic immunomodulatory mechanisms in humans remain poorly understood. To address this, this study aimed to investigate the impact of Bojungikki-tang on systemic immunity in patients with advanced non–small cell lung cancer receiving immunotherapy. We performed transcriptomic profiling of peripheral blood mononuclear cells obtained from 19 patients with non–small cell lung cancer in a randomized clinical trial, of whom 12 received Bojungikki-tang plus atezolizumab and seven received placebo plus atezolizumab. RNA sequencing and pathway-based analyses were performed to characterize treatment-associated transcriptional changes, supported by quantitative PCR validation. Pathway analysis revealed that co-treatment with Bojungikki-tang induced a distinct transcriptional program, characterized by the enrichment of $CD8^+$ T-cell activation, natural killer cell–mediated cytotoxicity, and type I/II interferon signaling. Longitudinal analysis further confirmed increased immune responses to tumor cells in the Bojungikki-tang group after treatment. Using machine learning–based classification models trained on quantitative PCR measurements, we identified a concise four-gene signature—downregulation of IL1R2 and ETS2 and upregulation of TUSC2 and S1PR2—that reliably discriminated patients treated with Bojungikki-tang with a mean F1 score of approximately 0.75. Collectively, these findings suggest that Bojungikki-tang augments immunotherapy-related

immune activation and nominate a peripheral blood mononuclear cell–derived immune signature panel that may serve as a noninvasive indicator for monitoring Bojungikki-tang-associated immune modulation in non–small cell lung cancer, supporting a mechanistic rationale for larger clinical validation.